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EFFECT OF CHLORINE ON STRESS ETHYLENE PRODUCTION

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TINGEY D. T., PETTIT N. and BARD L. *Effect of chlorine on stress ethylene production.* ENVIRONMENTAL AND EXPERIMENTAL BOTANY **18,** 61–66, 1978.—Several plant species were exposed to chlorine to determine whether stress ethylene was induced. Increasing the chlorine concentration or duration of exposure increased stress ethylene production until a maximum level was reached; either a higher concentration or longer duration decreased ethylene production below the maximum. The production of stress ethylene persisted less than 24 hr following exposure. Stress ethylene increased prior to visual injury but it was associated with visual injury. However, low concentrations of chlorine that did not cause visual injury elicited stress ethylene formation, suggesting that stress ethylene production is a more sensitive measure of chlorine stress than foliar injury.

INTRODUCTION

CHLORINE IS USED in many industrial and chemical processes where it may escape into the atmosphere and injure vegetation.[4,8,15,20] In 1971, 6% of the vegetation injury reports in Nev. Jersey were attributed to either chlorine or hydrogen chloride gas.[5] Most chlorine studies have focused on symptomatology, or chlorine doses that caused visual injury.[3,4,9]

Plants normally produce low levels of hormonal ethylene but when subjected to environmental stresses, ethylene production increases. Increased ethylene production following stress is frequently referred to as wound or stress-induced ethylene. Injured tissue is the site of the stress-induced ethylene production.[1] Studies with gamma radiation, tobacco mosaic virus, bacterial toxins and oxone showed that stress ethylene production was proportional to the amount of stress.[13,14,16,18]

The objectives of this study were to determine if stress ethylene production was a sensitive indicator of chlorine stress to plants, and the influence of chlorine dose and visual injury on stress ethylene production.

MATERIALS AND METHODS

In this study the plants (Table 1) were grown from seed except for azalea, coleus and ivy which were propagated by cuttings. The plants were cultured in a Jiffy mix: perlite* (1:2; v:v) medium in 10 cm diameter pots and grown in a greenhouse at day/night temperatures of $26 \pm 3°C$ and $18 \pm 2°C$, respectively. Sunlight was supplemented and the light period extended to 14 hr/day with light from HID sodium vapor lamps yielding an intensity of 350 to 600 $\mu E\, m^{-2}\, s^{-1}$ (400–700 nm) at plant height. A modified Hoagland's solution was applied daily to the plants.

Plant exposures were conducted in modified greenhouse exposure chambers[10] under the same environmental conditions in which the plants were

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grown. The plants were exposed to chlorine 4–6 weeks after seeding; the cuttings were of a similar size. Chlorine diluted in nitrogen was metered into the exposure chambers to achieve the desired concentration and measured coulometrically with a Mast* ozone meter. The meters were calibrated daily with known concentrations of chlorine from a permeation tube. The plant exposures were generally for 2 hr and the chlorine concentrations ranged from 0 to 1 $\mu l l^{-1}$. Immediately following exposure, each plant and pot was enclosed in individual 2 ml saran bags (approximately 4 l). A wire frame supported the bag to minimize foliar contact and provide a relatively constant volume.

*Table 1. Effect of chlorine on stress ethylene production and foliar injury**

Plant species	Ethylene % increase above control	Cl_2 conc for maximum C_2H_4 ($\mu l l^{-1}$)	Response† index	Maximum foliar injury
Raphanus sativus, L. Radish, Cherry Belle	60	0.30	200	70
Arachis, hypogaea, L. Peanut, Comet	83	0.44	189	6
Tagetes erecta, L. Marigold, Gold Galore	65	0.35	186	6
Lycopersicon esculentum, Mill. Tomato, Roma	61	0.54	113	32
Glycine max, (L.) Merr. Soybean, Dare	73	0.75	97	43
Phaseolus vulgaris, L. Bean, Pinto 111	69	0.87	79	23
Rhodendron obtusum, (Lindl.) Planch Azalea, Dogwood	73	0.97	75	0
Cucumis sativus, L. Cucumber, Bravo	70	1.00	70	16
Zinnia elegans, Jacq. Zinnia, Envy	48	0.90	53	27
Triticum aestivium, L. em Thell. Wheat, Hyslop	53	1.00	53	13
Coleus blumei, Benth. Coleus	51	1.00	51	7
Eucalyptus globulus, Labill. Eucalyptus, Blue Gum	51	1.00	51	11
Zea mays, L. Corn, Monarch Advance	22	0.44	50	27
Hedera helix, L. Ivy, English	27	0.90	30	0

*Plants were exposed for 2 hr and chlorine concentrations ranged from 0 to 1 $\mu l l^{-1}$. 45 plants of each species were used.

†The response index = % C_2H_4 increase/Cl_2 conc.

The encapsulated plants were incubated at 26°C for 3 hr in the dark. Preliminary studies with light and dark incubations showed no significant difference in stress ethylene production, therefore, dark incubations were chosen for consistency with previous studies.[18] Ethylene was sampled from the bag surrounding the plant by inserting a needle of a 1 ml glass syringe through a septum in the side of the bag and quantified as previously described except that a 2.4 m porapak N (80–100 mesh) column operated isothermally at 80°C was used.[18] There was no evidence of leaf wilt or visual injury when the ethylene was sampled. Approximately 72 hr following exposure, foliar injury was visually assessed in 5% increments as a percentage of the plant leaf area exhibiting injury. When the chlorophyll content was determined it was extracted 72 hr following exposure using 80% acetone and quantified as described by GOTTSHALK and MULLER.[7]

Earlier studies indicated that the variance of ethylene measurements increased with the ethylene concentration. Therefore, the data were transformed to their respective natural logarithms to stabilize the variability.[17] All statistical calculations were performed on the log-transformed data.

RESULTS

Stress ethylene production increased in all species tested with chlorine, however, three apparently different relationships between stress ethylene production and chlorine concentration were observed (Fig. 1). As shown in Fig. 1, the stress ethylene production in azalea increased above the control when the chlorine concentration exceeded 0.25 $\mu l\,l^{-1}$ chlorine and continued to increase over the remaining concentration range. In contrast, over the same chlorine concentration range ethylene production reached a plateau in soybean, while in radish the production rose to a maximum, then decreased.

Variability in chlorine-induced stress ethylene production was found in a wide range of plant species (Table 1). Chlorine increased stress ethylene production between 22 and 83% above control; maximum stress ethylene production occurred at chlorine concentrations between 0.3 and 1.0 $\mu l\,l^{-1}$. Since 1 $\mu l\,l^{-1}$ chlorine was the highest concentration used, it was not possible to determine whether in some species higher concentration might have elicited a greater increase in stress ethylene production. A chlorine response index for each species was computed by dividing its maximum percent increase in stress ethylene production by the chlorine concentration that elicited that maximum (Table 1). The response indices ranged from a low of 30 for English Ivy to a high of 200 for Radish. The higher the numeric index the more sensitive the plant, indicating that maximum stress ethylene production occurred at relatively low chlorine concentrations.

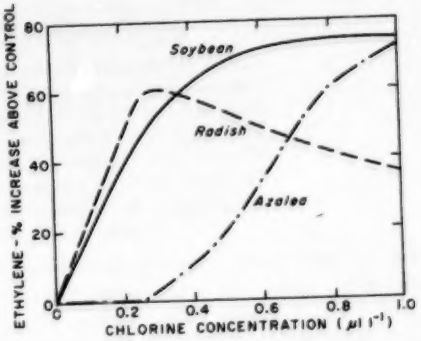


FIG. 1. The relationship between chlorine concentration and stress ethylene. Plants were exposed to a range of chlorine concentrations 0–1.0 $\mu l\,l^{-1}$ for 2 hr. A smooth curve was drawn through the data to illustrate the relationship. Each line was based on 45 observations.

To determine the influence of exposure duration on stress ethylene production, tomatoes and soybeans were exposed to 0, 0.25, or 0.5 $\mu l\,l^{-1}$ chlorine for 15–120 min. Chlorine exposures to tomatoes for 15 min increased stress ethylene production 65 and 58% above control and for 30 min 83 and 67% for the 0.25 and 0.5 $\mu l\,l^{-1}$ chlorine treatments, respectively. Longer exposures also increased stress ethylene above the control level but the concentrations were less than the maximum that occurred after 30 min. A similar trend was observed for soybeans.

Ethylene concentrations were measured immediately following exposure and hourly for 4 hr to determine the rate of ethylene accumulation in the bags (Fig. 2A). Ethylene accumulated in both

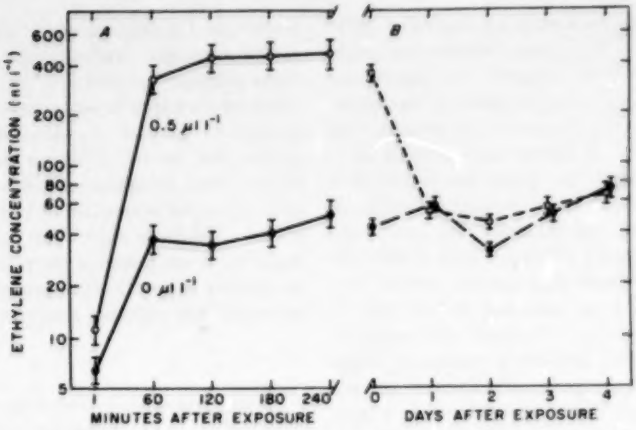


FIG. 2. The duration of stress ethylene production. Each mean is based on 6 observations and is shown with its standard error. (A) Tomatoes were exposed to 0 or 0.5 $\mu l\,l^{-1}$ chlorine for 2 hr and encapsulated immediately following exposure. Stress ethylene accumulation was sampled at 1, 60, 120, 180 and 240 min following exposure on each bagged plant. (B) Tomatoes were exposed to 0 or 0.5 $\mu l\,l^{-1}$ chlorine for 2 hr and then encapsulated and incubated for 3 hr. Plants were encapsulated only once either 0, 1, 2, 3, or 4 days after exposure.

the control and chlorine-exposed plants; however, significantly more ethylene accumulated in chlorine-exposed plants. The ethylene concentration rose rapidly during the first hour after exposure but as the time from the exposure termination increased the rate of ethylene accumulation decreased. The small increase in the control plants could have resulted from handling only.

To determine the duration of stress ethylene production, plants were exposed to chlorine for 2 hr and then encapsulated at various times, 0, 1, 2, 3, and 4 days after exposure. Each plant was encapsulated only once for 3 hr. The 0.5 $\mu l\,l^{-1}$ treatment significantly increased stress ethylene production on the day of exposure (Fig. 2B). However, within 24 hr stress-induced ethylene production had returned to the control level and remained there for the duration of the study.

A study was conducted to determine the relationship between stress ethylene production and visual injury. The chlorophyll content was used as a measure of visual injury. Stress ethylene production was measured immediately following exposure, while chlorophyll content was determined 72 hr later. In both soybean and tomato, the stress ethylene production was inversely related to chlorophyll concentration, indicating an association between the stress ethylene and foliar injury (Fig. 3). No visual leaf necrosis developed at chlorine concentrations up to and including 0.2 $\mu l\,l^{-1}$ on either soybean or tomato. Chlorine concentrations as low as 0.1 $\mu l\,l^{-1}$ for 2 hr significantly increased stress ethylene production (Fig. 4). This indicated that stress ethylene production occurred at low chlorine concentrations and in the absence of foliar injury.

DISCUSSION

The data presented in Fig. 1 showed three apparently different types of curves relating the percent increase in stress ethylene production to the chlorine concentration. The Arndt–Schulz law states that there is a universal tendency for low concentrations of toxicants to stimulate biological processes and for higher concentrations to depress them.[12] Also, the biological process is stimulated at lower toxicant concentrations in sensitive than tolerant species.[12] Radish, a chlorine sensitive species (Table 1), exhibited both the increase and decrease in stress ethylene production. While azalea and soybean, intermediate chlorine sen-

sitive species, showed only the stimulation of stress ethylene production, probably because the chlorine concentration was not sufficient to cause a depression. This curvilinear relationship between chlorine and stress ethylene production is substantially different than the apparent log-linear relationship of stress ethylene with increasing ozone concentration.[18]

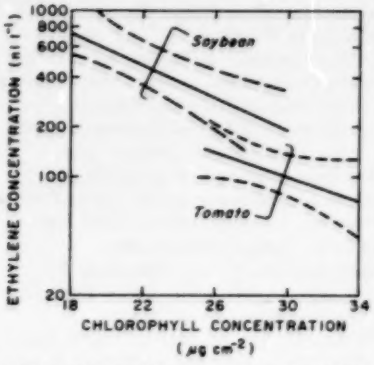


FIG. 3. The relationship between chlorophyll concentration and stress ethylene production. Plants were exposed to a range of chlorine concentrations 0–1 $\mu l\,l^{-1}$ for 2 hr. Stress ethylene was measured on the day of exposure following a 3 hr incubation and chlorophyll content was determined 72 hr after exposure. Chlorophyll was extracted from the total foliage of each plant. Each linear regression line based on 40 observations is shown with its 95% confidence limits. The slopes of the two linear regression lines for soybean and tomato were not statistically different.

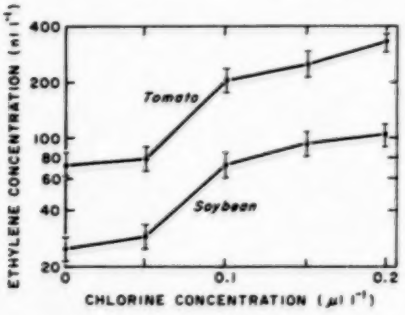


FIG. 4. Effect of sublethal chlorine concentrations on stress ethylene production. No visual injury developed on any plants. Each mean, based on 12 observations, is shown with its standard error.

The duration of stress ethylene production following a chlorine exposure was similar to that observed for ozone.[18] The maximum production of stress ethylene occurred within the first 2 hr following exposure; within 24 hr there was essentially no difference between exposed plants and control plants. The decrease in the rate of stress ethylene production following exposure suggested that, as the plants compensated for or repaired the chlorine injury stress ethylene production decreased. The burst of stress ethylene following a chlorine exposure indicates that ethylene is evoked as part of the plant's primary response mechanism to chlorine.

The sensitivity ranking of the plant species to chlorine as determined by the response index generally agreed with previous reports[3,6,9] using visual injury ranking techniques. However, in this study there were some differences in sensitivity from earlier studies probably because different plant cultivars were used.

The increase in stress ethylene could explain the increased leaf abscission, premature leaf drop, and epinasty associated with chlorine exposures.[3,19] Also, chlorine will increase the enzyme activities of peroxidase and polyphenoloxidase.[11] Ethylene is known to induce leaf abscission, epinasty, and activate peroxidase and polyphenoloxidase enzymes.[1]

Data from this study indicated an association between increased stress ethylene production and foliar injury (decreased chlorophyll). The similarity between the slopes relating stress ethylene production to decreased chlorophyll concentration for tomato and soybean suggested that stress ethylene production was dependent upon the damage to a finite number of cells rather than being species dependent. Also, concentrations of chlorine ($0.1\ \mu l\,l^{-1}$) that did not cause visual injury depressed photosynthesis[2] and induced stress ethylene production. Stress ethylene production occurred prior to the appearance of any visual injury and at chlorine levels below which any visual injury occurred. This suggested that stress ethylene was a sensitive indicator of chlorine stress on plants.

REFERENCES

1. ABELES F. B. (1973) *Ethylene in plant biology*. Academic Press. New York. 302 pp.

2. Bennett J. H. and Hill A. C. (1974) Acute inhibition of apparent photosynthesis by phytotoxic air pollutants. Pages 115–127 *in* M. Dugger ed. *Air pollution effects on plant growth*, ACS Symposium Series 3.
3. Brennan E., Leone I. and Daines R. H. (1965) Chlorine as a phytotoxic air pollutant. *Int. J. Air Wat. Pollut.* **9,** 791–797.
4. Brennan E., Leone I. and Holmes C. (1969) Accidental chlorine gas damage to vegetation. *Plant Dis. Rep.* **11,** 873–875.
5. Feliciano A. (1972) *1971 Survey and assessment of air pollution damage to vegetation in New Jersey*. EPA-R5-72-010. U.S. Environmental Protection Agency, Washington. 43 pp.
6. Gabriels R. (1973) Luchtpollutie: tolerantie van landen tuinvonevegewassen. *Meded Rijkssensier-plantent.* **29,** 1–35.
7. Gottshalk W. and Muller F. (1964) Quantitative Pigmentuntersuchungen an strahlen-induzierten Chlorophyllmutanten von *Pisum sativum*. *Planta* **61,** 259–282.
8. Harger J. R. E. (1973) Damage to vegetation by chlorine gas. *Int. J. Environ. Studies* **4,** 93–108.
9. Heck W. W., Daines R. H. and Hindawi I. J. (1970) Other phytotoxic pollutants. Pages F1–F24, *in* Jacobson J. S. and Hill A. C. eds. *Recognition of air pollution injury of vegetation: a pictorial atlas*. Air Pollution Control Association, Pittsburgh.
10. Heck W. W., Dunning J. A. and Johnson H. (1968) *Design of a simple plant exposure chamber*. DHEW, National Center of Air Pollution Control Publ. Washington. APTD–68–6.
11. Il'kun G. M., Pankrat'ev V. V., Tarasenko S. A., Mironova A. S. and Mikhailenko L. A. (1967) The sensitivity of plants to air pollution. *Puti Povyshenya Intensivnosti Prod.* Fotosin No. 2, 124–133. Cited in Chem. Abstracts 68, 71919, 1968.
12. Lamanna C. and Mallette M. F. (1965) *Basic Bacteriology. Its Biological and Chemical Background*. William and Wilkins, Baltimore, Md. 1001 pp.
13. Maxie E. C., Eaks I. L., Sommer N. F., Rae H. L. and El-Batal S. (1965) Effect of gamma radiation on rate of ethylene and carbon dioxide evolution by lemon fruit. *Plant Physiol.* **40,** 407–409.
14. Nakagaki Y., Hirai T. and Stahmann M. A. (1970) Ethylene production by detached leaves infected with tobacco mosaic virus. *Virology* **40,** 1–9.
15. National Research Council, Committee on Medical and Biological Effects of Environmental Pollutants. (1976) *Chlorine and hydrogen chloride*. National Academy of Science. Washington. 282 pp.
16. Shain L. and Wheeler H. (1975) Production of ethylene by oats resistant and susceptible to victorin. *Phytopath.* **65,** 88–89.
17. Snedecor G. W. and Cochran W. G. (1968) *Statistical methods*. 6th Edn. The Iowa State University Press. Ames. 593 pp.
18. Tingey D. T., Standley C. and Field R. W. (1976) Stress ethylene evolution: A measure of ozone effects on plants. *Atmospheric Environ.* **10,** 969–974.
19. Vaydik F. and Loncar M. (1955) The effects of air pollutants on vegetation in the Detroit–Windsor study area. *Air Pollut. Control Assoc. Proc. 48:* Paper No. 16.
20. Whenham G. R. (1969) *Report of the effects of chlorine gas release*. Veterinary Services Division. Edmonton Alberta, Canada.